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REVIEW ARTICLE

Knee Meniscus Injury: Insights on Tissue engineering Strategies Through Retrospective Analysis and In Silico Modeling

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Abstract | The present work focuses on three main aspects such as (1) extent of surgically managed human meniscal injury problem among the selected population (patients underwent meniscectomy), (2) viability of meniscus cells (isolated from surgical debri) at different age groups, and (3) simulation studies on model scaffolds to understand the biomechanical aspects. Thus, this study gives insights on the severity of the knee meniscus injury in the selected region and tissue engineering aspects which can in turn help in the design and development tissue-engineered construct for the regeneration of damaged tissue. Total 1025 patients (who underwent knee surgery related to meniscal injuries) data were collected from 2012 to 2015. With available additional data, main population was subdivided into two subpopulations. Retrospective and predictive statistical analyses using different statistical tools were performed for gender, age groups, causes of injury, symptoms, pre-treatment symptomatic duration, location of injury, and treatment given. Medium active persons with 23–28 BMI had shown more meniscus damage occurrences in subpopulation 2 ($n=316$). 125 meniscus surgical debrises were collected and cells were isolated from the samples to analyze the cell count in each age group. This study showed that as age increases, cell viability was found to be decreased. In this study using simulation studies, the stress distribution against varying applied load was analyzed using COMSOL MULTIPHYSICS (version 4.3b). For this study, two different model scaffold systems were used such as poly(methyl methacrylate) (PMMA) and silicone. This approach can be a strong foundation for the development implantable scaffolds with biomechanical properties comparable to human meniscus.

Keywords: *Meniscus injury, Meniscectomy, Epidemiological analysis, Cell viability, In silico modeling*

1 Introduction

Meniscus is a wedge-shaped cartilage present in between tibia and femur. It has a vital role in maintaining knee biomechanics by distributing the load and helping to withstand different forces like compression, tension, and shear. Human menisci tend to get damaged due to excessive physical or sports activities, trauma, etc. Amidst the various treatment modalities available for acute meniscal injuries, partial or total meniscectomy (removal of the damaged meniscus) is common. Repair through suturing like all inside-out

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technique is also performed when meniscus damage is in the vascular zone. About 10 million cases are reported in India for knee meniscus injuries every year.[1] In spite of high occurrences of knee meniscus problems, there are considerably fewer retrospective data available in India. John et al.[2] studied the occurrence of sports-related injuries in Indian athletes with a main focus on epidemiological and demographical studies (sample size 465, 5-year period). In non-athletic Indian population (sample size-192), Jacob and Oomen[3] reported that delay in presentation time for treatment increases the severity of meniscus tears and recommended early reconstruction to avoid further complications. Delay in presentation time also increases the complexity of meniscus tears such as knee instability and dysfunction with time.[4] Despite of these reports, till date, there is no statistical survey on surgically managed human meniscus tear with respect to South Indian population. The previous reported studies have been limited to occupational knee meniscus injuries. To understand the severity of the problems associated with the population underwent knee meniscal surgery, a retrospective study in this area is required.

An alternative strategy for the conventional treatment is to develop tissue-engineered constructs which regenerate the tissue. For this, different natural and synthetic polymers have been used by researchers to develop meniscus scaffold such as collagen, gelatin, hyaluronic acid, polycaprolactone, silicone, poly (methyl methacrylate) (PMMA), etc.[5] Mimicking the biomechanical function of the knee meniscus tissue still remains a challenge and requires deep research in this area to develop a successful implant. Mahar et al.[6] suggested that, for the development of meniscal substitutes, integration of in silico modeling studies is as important as in vitro and in vivo studies. The main in silico modeling softwares available for research purpose are Ansys, COMSOL MULTIPHYSICS, etc. Biomechanical aspects of scaffolds can be stimulated using these softwares at different stress conditions, at different positions of knee, and at various angles of force. These studies enable researchers to develop functional knee meniscus tissue-engineered constructs.

The main objectives of this study are the following: (1) to understand extent of surgically managed symptomatic knee meniscus problem in the South Indian population, especially in Coimbatore region. Retrospective and predictive statistical analyses were performed on these data to analyze the problem using different statistical tools. To our knowledge, this is the first statistical study which addresses surgically managed meniscus injury status of such patients; (2) viability of meniscus cells (isolated from surgical debri) at different age groups; and (3) simulation studies on model scaffolds to understand the biomechanical aspects. The objective of this study is to analyze the stress developed on the meniscal scaffolds by simulating it using COMSOL Multiphysics 4.3b.

2 Experimental Method

2.1 Statistical Analysis of Collected Data

2.1.1 Data Collection

Data pertaining to surgically managed meniscus injuries were collected at Coimbatore region, India from two leading ortho-based hospitals namely, Ganga hospital (referral centre for orthopedics) and Ortho One Orthopaedic Speciality Centre (specialized in sports medicine). All data were collected after approval from Institutional Human Ethical Committee (No: 12/193, January 2013) and due permission from the hospitals. Data were collected from the hospital records of the patients who underwent knee surgery during 01-January-2012 to 31-December-2015 from the above-mentioned hospitals. All the patients were hailed from various parts of South India mainly from Tamilnadu, Kerala, Karnataka, and Andhra Pradesh. The inclusion criteria were selected as: patients underwent meniscus injury related surgery (including meniscus injury associated with cruciate ligament tear) like meniscectomy partial or total and meniscus repair. The exclusion criteria included any other knee-related surgeries where meniscus injury was not involved, such as total knee replacement, articular cartilage damage, and any other injury in the knee. Data (total 1025) were collected and segregated based on patients records present in the respective hospitals during January 2012–December 2015.

2.1.2 Study Design

The selected population had acute knee problem which required surgical intervention. The data common for both hospitals were age, gender, duration of pre-treatment symptom, and surgical procedure performed. Among the 1025 patient data, 709 and 316 were collected from Ganga hospital and Ortho One Orthopaedic Speciality Centre, respectively. The main population was classified based on available and additional data (Fig. 1). The parameters like age, gender, cause of injury, duration of problem, type of meniscus and knee affected, and treatment provided were available for all 1025 data and were considered as main data. Apart from these parameters, Ganga hospital data (subpopulation 1) contained type of tear, whereas Ortho One Orthopaedic Speciality Centre data (subpopulation 2) had height, weight, and profession as additional data (Fig. 1).

The parameters were further stratified to study in depth. For this, gender was classified into male and female. The age group was categorized as < 20, 20–30, 30–40, 40–50, and > 60 years. The pre-treatment symptomatic duration was divided to less than 1 year to above 6 years to study the severity of injury. The main etiological factors recorded were sports, road accidents (two-wheeler (TW-RTA), and four-wheeler road accidents (4W-RTA), trivial fall from height and others like twisting injury, degenerative, combination of multiple factors and unknown disorders. Main symptoms present in patients having meniscus tear were severe pain, locking, knee instability, and swelling. All symptoms were associated with pain and some patients had pain alone without any other symptoms. Meniscus tear were present in left or right knee and in few cases, in both the knees. Medial (MM) and lateral (LM) meniscus injuries associated with or without anterior cruciate ligament (ACL) tear were also considered for statistical analysis. The treatments given for patients were meniscectomy [total (TM)/partial (PM)], repair (suturing and all inside-out techniques), and PM along with repair.

The type of tears was classified as horizontal (HT), flap tear (FT), bucket handled (BT), oblique tear (OT), complex tear (CT), and others. Others include radial (RA), flap (FT), vertical (VT), longitudinal (LT), root (RT), horizontal with flap (HT-FP), radial with horizontal (R-HT), vertical with oblique (V-OB), and horizontal with oblique (HT-OB) tear. Based on the height and weight data, body mass index (BMI) was calculated for the analysis using online calculator created by National Institute of Health (NIH), USA.[7] The total BMI range (13–63) was subdivided according to NIH classification as underweight, normal weight, overweight, and obese, having BMI of < 18.5, 18.5–24.9, 25–29.9, and > 30, respectively. The profession details of patients were classified as high active, medium active and less active population[8] as given in Table 1.

To evaluate the subpopulations as a true representative of main population, Chi-square goodness of fit test was performed with selected three major criteria such as gender, age group, and cause of injury using Minitab 17 software. During this analysis, data in each criterion (main population in proportion) were compared with the subpopulation (observed and expected), and then, the test was performed for the Chi-square contribution analysis with the null hypothesis (H_0)

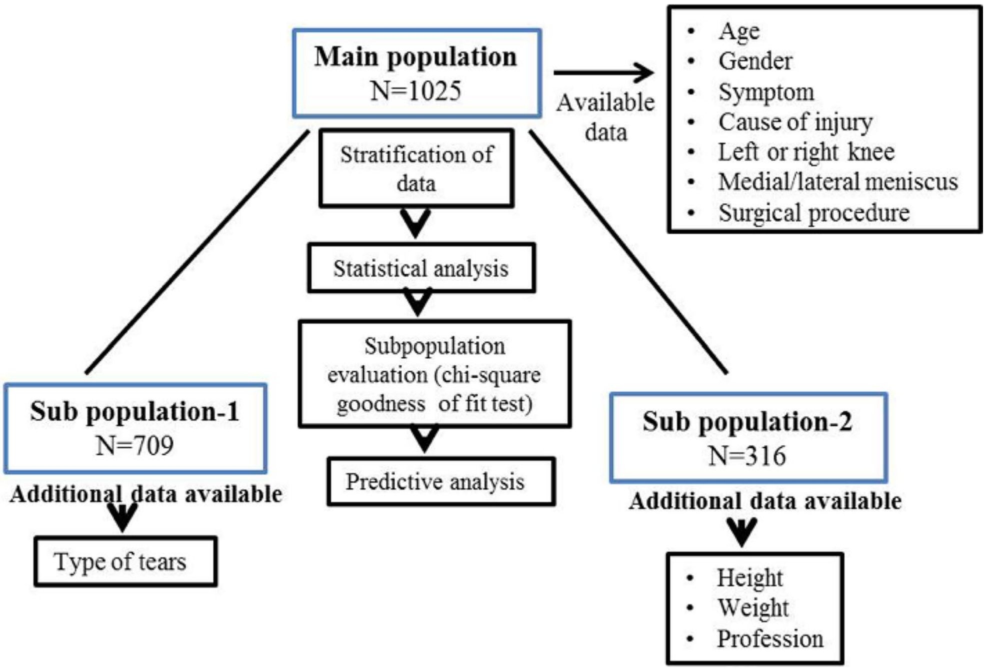

Figure 1: Flowchart on patient data details and statistics analysis.

Table 1: Classification of activity based on profession.

Activity level	Profession
High active	Students, army, police, navy officials, construction worker, carpenter, mechanic, plumber, sports person
Medium active	Doctor, IPS, business, mechanical engineer, speech pathologist, security, lab assistant, assistant professor, teacher, housewife
Less active	Software Engineer, shop owner, lawyer, apartment owner, officer, ceo, chit fund manager, banker, manager

"There is no difference between subpopulation and main population for the selected parameter". A P value of ≤ 0.01 was considered to be statistically significant (null hypothesis rejected). The frequency of occurrence ($F\%$) in particular age group was calculated as per Eq. 1 for the analysis of variation between age groups:

$$\text{F\% in age groups} = \left(\frac{\text{Total number of occurrence in an age group}}{\text{Total population in the age group}}\right) \times 100. \quad (1)$$

For all other statistical analysis, Origin Pro 8 was used.

2.1.3 Cell Count Analysis Isolated from Human Meniscus

Human knee menisci surgical debris were obtained from PSG Institute of Medical Sciences and Research and Ortho One Orthopaedic and Specialty Centre, Coimbatore, India, respectively (Ethical committee clearance No: 12/193 dated 24.01.2013) from the patients who underwent meniscectomy. The collected tissues of known size were subjected to surface sterilization and finely chopped using surgical knife. Furthermore, tissues were treated with trypsin, followed by collagenase and incubated until complete cells were released from the tissue. The released cells were cultured subjected under standard sterile conditions in vitro in Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum and 1% antibiotics, at 37 °C and 5% CO_2 was provided. The isolated cells were allowed to attach in the cell culture dishes, and at the end of day 5, cells were trypsinized and cell count was performed using trypan blue staining.[9]

2.1.4 Simulation of Stress Distribution Analysis for Meniscus Scaffolds

The modeling and stress distribution analysis was performed using COMSOL MULTIPHYSICS (version 4.3b). For the construction of femur bone model and meniscus model software such as CAD and MESHLAB, respectively, were used. Meniscus scaffold model was designed using two different materials such as PMMA and silicone with number of elements: tetrahedral 2429, triangular 1264, edge 112 and vertex 10; element volume ratio 3.786×10^{-4}; and mesh volume of 1878 mm^3.

In this study, force applied in different directions which is responsible for stress development was considered. Meniscus undergoes 45–99% compression during various physical activities. Body weight of an individual is concentrated in multiples of the actual weight depending upon the physical activity carried out. The entire anterior surface of the meniscus was subjected to force due to the femoral condyles. Thus, boundary load was applied on the surface, i.e., in the Z direction for analysis. Meniscus was also subjected to force from X and Y directions due to the ligaments attachment, and hence, force was applied from those directions and the results were recorded. The Z axis value was given according to the body weight of an individual. The equivalent force applied on the meniscus was calculated by multiplying body weight with earth's gravity using the following equation:

$$F = \text{weight} \times \text{gravity}. \quad (2)$$

The equivalent force exerted for a 40 kg person is 392 N (approximately 400 N). Different body weight (in kilograms) such as 40, 50, 60, 70, and 80 were considered for this study.

3 Results

Surgically treated meniscus damage was found to be higher in right knee ($n = 551$) compared to left and both knee ($n = 469$ and $n = 5$, respectively) in the total study population. Meniscal tears commonly occur in combination with anterior cruciate ligaments (ACL) tears. Among the meniscus tears (medial or lateral), occurrence of medial meniscus (MM) tear ($n = 529$, MM and MM and ACL combined) was found to be predominant followed by lateral meniscus (LM) injury ($n = 364$, LM and LM and ACL) (Fig. 2b).

In 132 patients, both the menisci were severely affected. Treatments like meniscectomy (partial or total) and repair were used for alleviating meniscus tear-related symptoms (Fig. 2c). Partial meniscectomy (PM) (86.82%) was the treatment given to the majority of patients irrespective of age. Other treatments like PM along with repair (7.91%), repair alone (4.69%), and total meniscectomy (0.59%) were also given depending upon type and severity of tear (Fig. 2c). It was found that MM injury was more in all age groups (20–60) except above 60 (Fig. 2d). In case of partial meniscectomy, the frequency of occurrence was found to be higher than other surgical procedures irrespective of age (Fig. 2e). 246 patients out of 1025 had knee instability which was not associated with ACL tear. Among 246 patients 13 patients were having both lateral and medial meniscus injury.

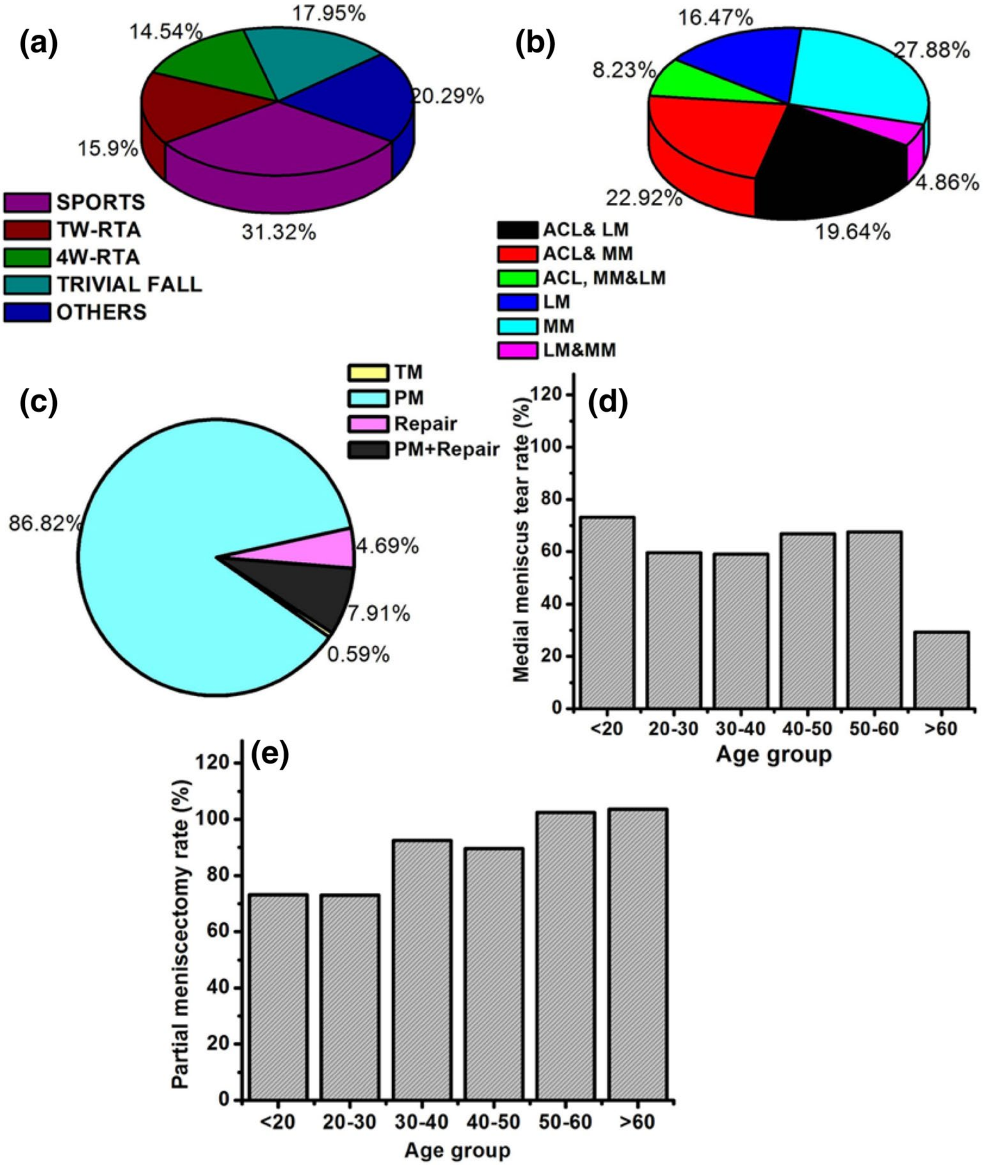

Figure 2: **a** Pie chart indicating the percentage of activity leading to meniscus injury (*n*=1025) (two-wheeler road accidents—TW-RTA; four-wheeler road accidents—4 W-RTA) (*n*=1025), **b** injury rate in lateral or medial meniscus or both (medial meniscus—MM; lateral meniscus—LM; anterior cruciate ligament—ACL) and c surgical procedure performed in patients with meniscal injury (total meniscectomy—TM; partial meniscectomy—PM) (*n*=1025); Frequency of **d** medial meniscus injury and **e** partial meniscectomy in different age groups (*n*=1025).

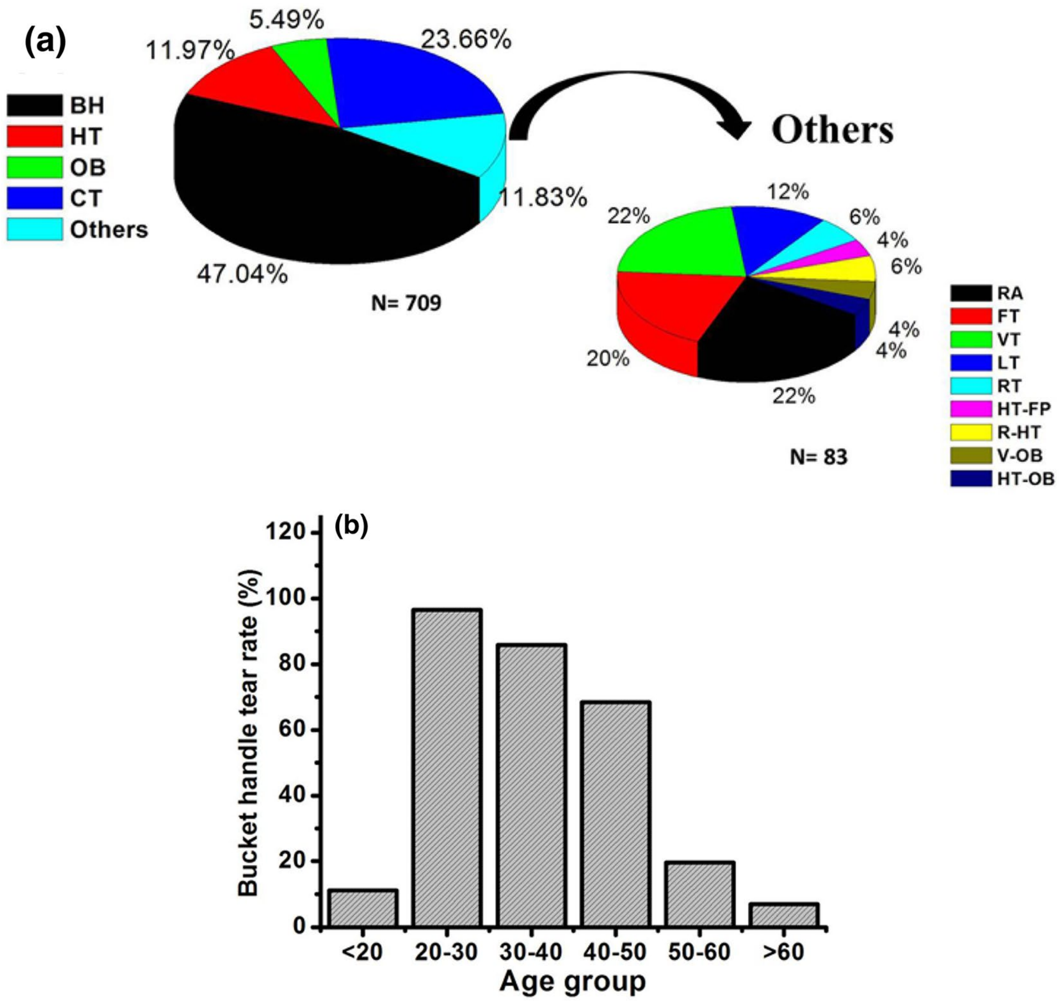

Figure 3: **a** Incident rate of different meniscus tears such as bucket handled (BH), horizontal (HT), oblique (OB), complex tear (CT), and other tears. Other tear includes radial (RA), flap (FA), vertical (VT), lateral (LT), root (RT), and the combination of different tears ($n = 709$); **b** frequency of occurrence of bucket-handled tear at different age groups ($n = 709$).

3.1 Characteristics of Subpopulation Data 1 (n = 709)

The subpopulation 1 ($n = 709$) had additional data on occurrence of different types of tears leading to surgery. Frequency distribution of different types of tears is shown in Fig. 3a. Bucket-handled (BH) tear had higher frequency (47.04%) when compared to CT (23.66%), HT (11.97%), and OT (5.49%), and other (11.83%) tears. The other tears were classified as radial (RA), flap (FA), vertical (VT), lateral (LT), and root (RT). The combination of meniscus tears was also present. The occurrence of combination of tears was less as compared to frequency of single tears. From the collected data, horizontal with flap tear ($n = 2$), radial with horizontal tear ($n = 2$), vertical with oblique tear ($n = 2$), and horizontal with oblique tear ($n = 2$) were also diagnosed (Fig. 3a). The frequency of occurrence for BH tear was found to be high in age groups between 20 and 50 years (Fig. 3b). Surgical treatments given to the patients depending upon different tear types are shown in Fig. 3 which confirms that the major surgically managed tear is BH.

Table 2: Chi-square contribution for gender.

Category	Observed	Proportion	Expected	Chi-square contribution
Male	608	0.815	578.2	1.5
Female	101	0.18	130.7	6.7

Table 3: Chi square contribution for age group

Category	Observed	Proportion	Expected	Chi-square contribution
< 20	70	0.07	75.17	0.35
20–30	352	0.26	271.79	23.67
30–40	290	0.25	260.22	3.40
40–50	208	0.26	276.12	16.80
50–60	73	0.10	102.64	8.56
> 60	32	0.03	39.03	1.2

Table 4 Chi square contribution for cause of injury

Category	Observed	Proportion	Expected	Chi-square contribution
Sports	198	0.31	222.0	2.6
Accidents	302	0.30	215.8	34.4
Trivial fall	166	0.17	127.2	11.7
Others	43	0.20	143.8	70.7

3.1.1 Goodness of Fit for Subpopulation 1

For the evaluation of goodness of fit for subpopulation 1 to main population, Chi-square test was performed for gender, age group, and causes of injury. In case of gender, Chi-square contribution was found to be high for female, while the male population was found to be the true representative of main population (Table 2). In case of age groups (Table 3), null hypothesis was rejected because of high Chi-square contribution from < 20 to 40–50 age groups. Furthermore, with the step-wise elimination of these two age groups, all other age groups (20–30, 30–40, 50–60, and > 60) were found to be true representative of the main population. Table 4 shows the Chi-square contribution for the causes of injury for subpopulation 1. The *P* value was found to be 0.000, and hence, null hypothesis was rejected. Chi-square contribution was highest (70.7) for the category 'Others'. Hence, we excluded the value and ran the test further. Finally, the data category of 20–60 age group male population, accidents, and trivial fall category were found to be the true representative of the main population.

3.2 Characteristics of Subpopulation Data 2 (*n* = 316)

Data on patients' height, weight, and profession were available for 316 patients (265 males and 51 females) collected from Ortho One Orthopaedic Specialty Center, Coimbatore. Considering severe meniscus injury rate vs. activity level, high frequency of surgically managed meniscus injuries was observed in medium active population ($n = 190$) compared to low active: ($n = 60$) and high active ($n = 66$). For medium active population, the risk was highest for 20–30 age groups and the frequency was declining with increase in age group above 20–30 (Fig. 4a). In case of high and low active population, the maximum occurrence was observed in age group of 30–40. Figure 4b shows the number of patients treated due to various causes of injuries with respect to their BMI. The patients having BMI in the range of

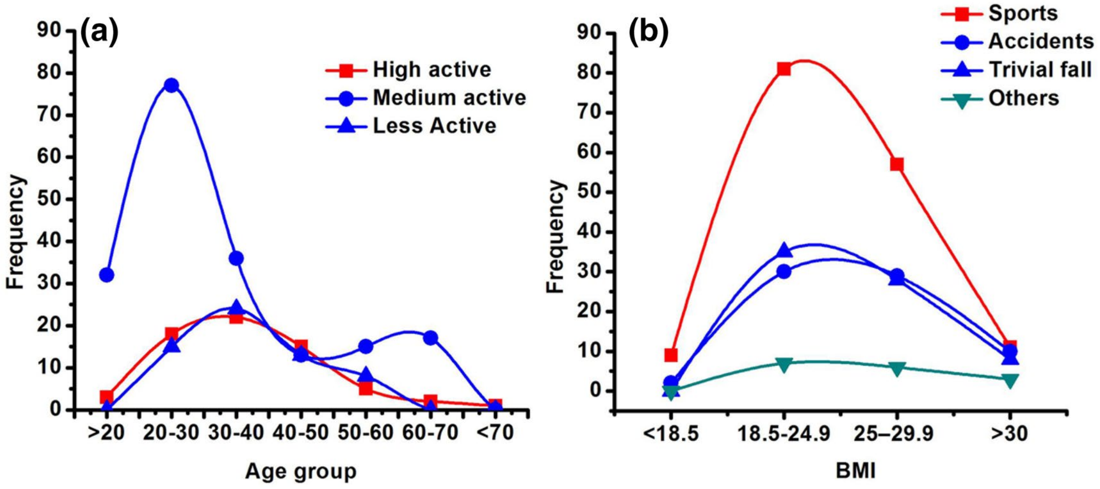

Figure 4: Meniscus injury frequency based on **a** age group and **b** BMI ($n = 316$).

Table 5: Chi-square test—age group.

Category	Observed	Proportion	Expected	Chi-square contribution
< 20	35	0.06	21.58	8.3
20–30	110	0.34	108.52	0.02
30–40	82	0.28	89.41	0.61
40–50	41	0.20	64.13	8.3
50–60	28	0.07	22.51	1.3
> 60	20	0.03	9.87	10.4

23–28 were found to be more affected irrespective of the cause of injury (Fig. 4b).

3.2.1 Goodness of Fit for Subpopulation 2

Subpopulation 2 ($n=316$) was studied to understand the significance level for true representation of main population ($n=1025$). Initially, gender-wise Chi-square fitness test was performed which confirmed that the subpopulation (i.e., $n=316$) was a true representative of main population ($n=1025$). Furthermore, goodness of fit test was performed for symptomatic meniscus injury rate in different age groups where 20–60 age groups were found have a P value of 0.11 (Table 5). This indicates that the data collected from subpopulation in this age group (20–60) are true representation of the main population, while the patient's data collected for age groups <20 and >60 were more than the expected number from subpopulation 2.

Similarly, for cause of injury, P value was less than 0.01, and hence, null hypothesis were rejected. The high contributing Chi-square values for sports and others (Table 6) were removed and significance test was repeated. Finally, it was concluded that the 120-patient data from the subpopulation 2 ($n=316$) having 20–60 age group in both genders with causes like accidents and trivial fall were found to be the true representative of the main population ($n=1024$).

3.3 Cell Count Variation at Different Age Groups

Figure 5 shows the isolation and attachment of knee meniscus primary cells in the in vitro standard conditions (Fig. 5a) and cell count variation profile at different age groups (Fig. 5b). The samples were segregated into different age groups ($^{<}20$–<60) and analyzed the cell count from the collected meniscus samples. Cell viability and proliferation were found to be in a decreasing trend as age increases. Isolated cells at the age group of less than 20 showed the highest proliferation rate among all other age groups with a cell count of $3.5\pm0.28\times10^4$ cells/ml. However, in all other age groups (20–30, 30–40, 40–50, and above 60), the cell count was in the range of 2.7–0.51×10^4 cells/ml (Fig. 5b). This confirms that the potential source of cells for meniscus tissue engineering can be mesenchymal stem cells isolated from patients as primary cell viability and proliferation ability decreases as age increases.

3.4 Simulation Model for Developing Tissue-Engineered Construct

Figure 6a shows the different directions of force experienced inside meniscus. When these forces exerted in the knee meniscus, it deforms radially and circumferentially which is mostly prevented by the horns. Sudden twisting due to vigorous sports activities or accidents are the main reason of meniscus damage. The incidence of LM injury was found to be more common than MM injury; in this study, LM meniscus model was designed to analyze the stress pattern at different directions. To mimic the actual condition of knee meniscus force environment, femur model was designed. In this study, the stress profile of lateral meniscus was considered for the stress simulation. To simlate the knee joint, half of the femur bone was designed and was used to understand the different stress profiles exerted in the meniscus tissue.

Figure 6c– e refers to the different directional force applied to the knee meniscus to observe the stress profiling. From this study, it was clear that, when the load was applied from all the three directions, more stress was found to be developed in the meniscus when compared to other loading conditions. Furthermore, the stress profiling of two different scaffold models such as PMMA and silicone was performed. Figure 6 f and g represents the stress profiling of silicone and PMMA, respectively, for an applied force of 800 N in the Z axis and X axis. This study confirms that the silicone material was found to be having more stress

Table 6: Chi-square test for cause of injury.

Category	Observed	Proportion	Expected	-quare contribution
Sports	173	0.31	98.9	55.3
Accidents	81	0.30	96.18	2.39
Trivial fall	48	0.17	56.72	1.3
Others	14	0.20	64.12	39.1

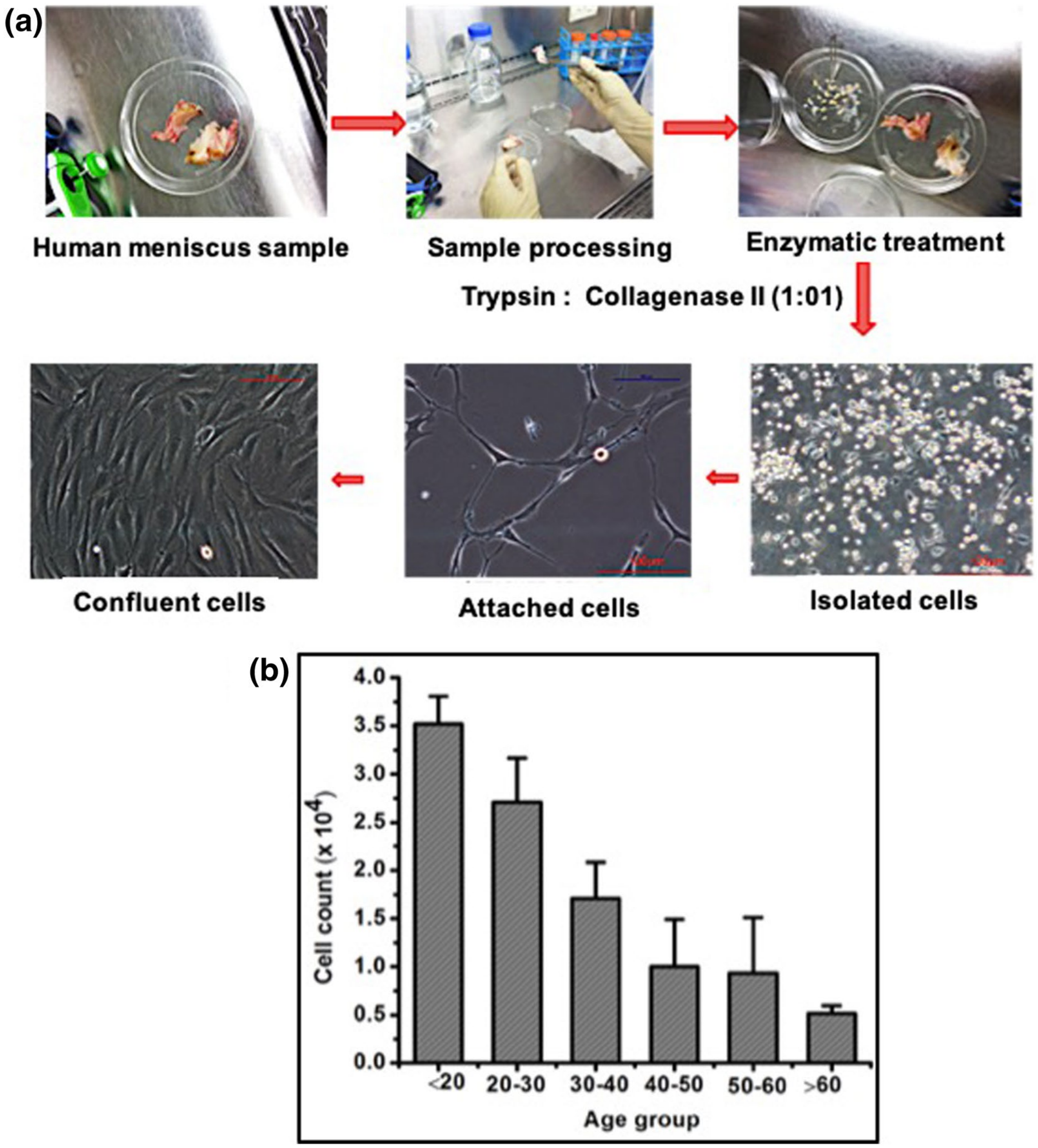

Figure 5: **a** Representative image of isolation of primary meniscus cells from the surgical debris, and **b** cell count of primary meniscus cells isolated from surgical debris (mean ± SD).

resistance compared to PMMA. The peak stress values developed for silicone and PMMA were 3.4 kPa and 3.6 kPa, respectively.

In another condition, the force exerted in the implanted scaffold at different human posture was studied. Table 7 represents the stress developed in different scaffolds (PMMA and silicone) at different applied force. The values given for *X* and *Y* axes represent different posture of the person (Table 7). From this study, it was clear that, depending upon the direction of force applied, different pattern of stress was developed in the scaffolds. However, the region where the stress developed remains constant, only amount of stress developed was found to vary. From the Table 7, it was clear that stress developed in silicone was less when compared PMMA as used as a scaffold model.

4 Discussion

The population selected for this study was the patients who underwent surgical intervention as mode of treatment. In Fig. 1d, medial meniscus injury rate was higher in all age groups studied except in above 60 years. In case of treatment given, incidence of PM was found to be more in all age groups. Thus, young aged patients may be prone to osteoarthritis in long run when

Table 7: Maximum stress developed in the meniscus scaffolds at different applied force.

Z axis	*X* axis	*Y* axis	Silicone (stress) kPa	PMMA (stress) kPa
400	100	0	1	1
400	– 200	0	2	2
400	200	0	1.1	1.2
400	– 300	0	2.5	2.6
400	0	– 300	1.7	1.7
400	400	– 300	2.4	2.4
400	300	400	2.6	2.9
400	400	0	1.6	1.8
400	300	0	1.4	1.5
400	0	400	2.5	2.6
500	0	0	1.4	1.3
500	200	0	1.3	1.4
500	– 200	0	2.3	2.3
500	400	0	1.8	1.9
500	– 400	0	3.3	3.4
500	0	200	1.9	2
500	0	– 200	1.6	1.6
600	400	200	2.5	2.5
600	– 400	200	3.6	3.7
600	– 400	– 200	3.7	3.7
600	400	0	1.9	2.1
600	– 400	0	3.6	3.6
600	600	0	2.5	2.7
700	200	200	2.4	2.6
700	200	0	1.8	1.9
700	400	200	2.7	2.9
700	0	– 400	2.6	2.6
700	0	300	2.8	2.9
700	– 400	– 200	3.9	4
800	400	0	2.3	2.4
800	800	0	3.3	3.6
800	0	– 300	2.5	2.6
800	0	– 600	3.5	3.5
800	800	400	4.3	4.6
800	800	– 400	4.2	4.3
800	800	– 600	4.8	4.8
800	1200	0	5.1	4.8

compared to elder patients (50–60 years). As reported earlier and also from Fig. 2b, c, MM injury and PM have the major occurrence considering the injury and treatments. However, there is no such evidence that this is true among all age groups. Hence, to understand the occurrence rate among different age groups, a predictive statistical analysis was performed. In this analysis, selected factor (MM tear or PM) was considered as major contributor if the occurrence rate was above 50%. From Fig. 1d, it can be concluded that up to 60 years, MM injury has major occurrence and was most common cause for meniscal damage. Beyond that, the argument "medial meniscal injury is higher than lateral" may not be true. However, in case of PM (Fig. 1e), proportional percentage was above 50% among all the age groups which indicates that, irrespective of age, PM was given as major treatment to alleviate meniscus injury-associated symptoms.

Different types of tears observed in subpopulation 1 ($n = 709$) patient data. The maximum number of tear frequency observed were BH tear followed by CT and HT. Depending upon different types of tears, the clinical manifestations also vary. BH tears were reported to mostly associated with knee instability and locking of the knee. This is due to the damage of entire inner region of the meniscus. The sudden extreme flexion of knee causes meniscus to be stuck inside the knee joint which leads to the damage.[10]

For analyzing whether this population is a true representative of main population, Chi-square goodness of fit test was performed for gender, age groups, and cause of injury. Male patients and age group 20–30, 30–40, 50–60, and above 60 populations were found to be the true representative of the main population. Accident and trivial fall incidence in subpopulation 1 were found to be the true representation of the main population. It may be argued that, as the subpopulation 2 is from a sports specialty hospital, their contribution to the main study population de-signifies the sport injury contribution of subpopulation 1. *P* value was found to be 0.000, and hence, null hypothesis was rejected, and Chi-square contribution was highest (70.7) for the category 'Others'. Hence, we excluded the value and run the test further. Finally, male population, 20–60 age group, accidents, and trivial fall category were found to be the true representative of the main population.

Subpopulation 2 was classified according to the profession and BMI. In this study, out of 316 patients, 160 cases were meniscus damage occurred due to sports. Among these 160 cases, only 34 people were highly active or sports men. Remaining 126 people were medium or less active, but the damage of meniscus was due to sports activity. This indicates that rather than a trained personal, amateurs are more prone to sudden twisting or hyperflexion of knee leading

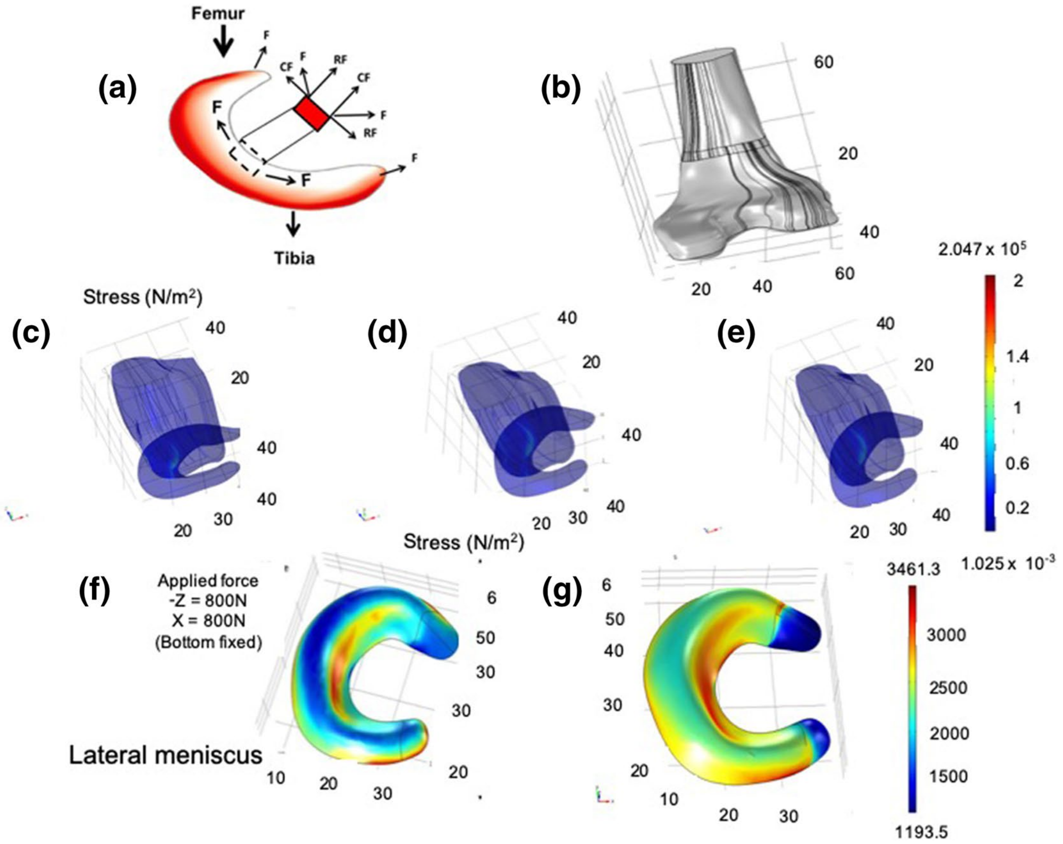

Figure 6: **a** Direction of force dissipated in the knee meniscus, **b** femur bone model constructed in CAD software; testing of meniscus deformation at different load levels, **c** force applied from the top of the lateral knee meniscus, **d** force applied in the *Z* and *X* directions, **e** force applied from the all the three directions; stress development studies on the meniscus scaffold models, **f** silicone, and **g** PMMA.

to damage of meniscus.[11] However, in case of highly active group such as sports persons, army or navy officials, they have proper coaching for the sports activities in which they participate. Hence, the rate of sports injury was comparatively less. As observed from Fig. 4a, the medium active population in the age group of 20–30 years is mostly affected, while in high and less active group, maximum damage has been observed in 30–40 years. This clearly indicates that the high active people like sports man has higher risk of getting meniscal damage at later stage of active life. According to NIH, underweight, normal weight, overweight, and obese have BMI of < 18.5, 18.5–24.9, 25–29.9, and > 30 respectively. The predictive analysis for meniscus injury rate was calculated for different BMI and based on activity level of patients. In case of activity level, medium active population had high risk of meniscus problems and the main cause was sports. However, our results were not in agreement with this finding. Statistical analysis was performed in the subpopulation 2 ($n = 317$), considering factors like BMI and profession. From the results, it was clearly evident that patients having BMI 23–28 at the age of 20–30 are more prone to meniscus damage (Fig. 4b). This directly correlates to the activeness of the person and knee meniscus damage is proportional to each other. The major reason of knee injury was found to be sports-related followed by vehicular accidents. This suggests that people in this age group are not only are at risk for acute meniscal tears, but also are prone for the accelerated progression of degenerative joint changes after injury. This indicates that rather than a trained personal, amateurs are more prone to sudden twisting or hyper flexion of knee leading to damage of meniscus.[11] However, in case of highly active group such as sports persons, army, or navy officials, they have proper coaching for the sports activities in which they participate. Hence, the rate of sports injury was comparatively less. Weiss reported that increase in BMI can increase the stress in knee joint during flexion which can lead to meniscus injuries.[12] If patients with BMI above 25 participate in sports, the chances of mechanical damage of meniscus are more when compared to patients of BMI level of below 24.9.[13] Obesity has long been recognized as a risk factor for knee osteoarthritis due to excessive loading of weight-bearing joints.[14] The possible cause of meniscus injury may be the life style changes.

From the retrospective analysis, it was evident that PM is the major treatment performed for patients. BH tear was found to have highest incidence compared to all other treatments. In this context, regeneration of functional tissue is the need of hour. Tissue engineering strategy can be a potential alternative treatment for meniscus tissue regeneration. In this using appropriate cells and biomaterials 3D biodegradable, biocompatible constructs can be prepared. Along with this, suitable growth factors help to enhance the regeneration of tissue which will be in an implantable form.

Different types of cells are available for knee meniscus tissue engineering such as meniscus cells, fibrochondrocytes, mesenchymal stem cells, and induced pluripotent stem cells are widely used. Human meniscus cells are considered as potential cell source for the regeneration of meniscus. However, due to aging, meniscus cells tend to reduce cellular viability.[15] Reduction in the volume of extracellular matrix (ECM) such as collagen and aggrecan due to applied load eventually makes the tissue non-regenerative due to loss of cellularity and lack of blood vessels. In this study, the cells isolated from different age groups who underwent meniscectomy prove that, as age increases, the viability profile of cells shows a decreasing proliferation trend. These findings are in line with the previous studies reported.[15–17] This makes regeneration of tissue more challenging in terms of age. The growth and proliferation rate of cells can be enhanced by signaling molecules such as growth factors, biomolecules, etc. Pillai et al.[9] developed an affordable unique combination of biomolecule supplementation medium (UCM) for the enhancement of cell proliferation and ECM synthesis. This UCM can enhance cell proliferation without altering cellular physical or biological properties. One of the main challenges in the development of meniscus scaffolds is to match the biomechanical characteristics of native tissue.

In silico models can help to develop tissues with functional properties comparable to human meniscus. In these studied models, pressure distribution against applied load can be analyzed. The main aim of the simulation study was to identify the stress distribution and localized stress concentration points (or zones). The values of x, y, and z axes stress differ from actual incidents. However, the load given at different directions are selected at different ratios which signify the change in resultant load vectors at different mesh elements to understand the stress distribution. This approach can be a strong foundation for the development implantable scaffolds with biomechanical properties similar to human meniscus. In this study, two different materials were selected to analyze the stress distribution against applied load. For this, different forces acting on knee meniscus at different directions should be considered. Meniscus experiences tensile, compressive, and shear forces that are generated during loading and unloading. Due to these forces, meniscus deforms radially (RF) and circumferentially (CF) which is resisted by the anterior and posterior horns of the meniscus. This resistance causes hoop stress, compression, radial tension, and shear due to the collagen and proteoglycan arrangement within the tissue which helps to prevent the damage or tear.[18] The radial, circumferential, and random arrangement of collagen helps in resisting radial tension and hoop stress. Researchers have been reported the knee meniscus compressive strength. In a study performed by Chia et al.[19], radial and axial compressive moduli of the human meniscus at 12% strain were found to be 76.1 kPa and 83.4 kPa, respectively. Even for the development for meniscus tissue-engineered scaffolds using polymers such as polycaprolactone[20, 21] and silk fibroin-polyvinyl alcohol [22], the compressive moduli at 12% strain were found to be 6 kPa,[19, 20] 190 kPa,[21] and 115 kPa.[22] Thus, this study suggests that the selection of biomaterial is a crucial step in development scaffolds for load bearing applications to match the mechanical properties of the native tissue. The model biomaterials selected for this study; silicone and PMMA were also found to show comparable maximum stress developed in the scaffolds to the human knee meniscus tissue.

Meniscus tears occur mainly because of axial and rotational forces due to sudden twisting. Thus, removal of meniscus partially or totally increases stress due to the load per unit of contact area which is approximately 200–300% high as compared to normal. This contributes to the damage of underlying articular cartilage and bone which leads to osteoarthritis. Guo et al. performed finite-elemental model analysis by developing biphasic 2D axisymmetric model. They found that 60% of the total load was carried by the fluid surrounded by the knee joint and maximum strain in the articular cartilage was found to be more than 350%. This proves that the patient after meniscectomy can be predisposed with osteoarthritis in long run. [23]

The scaffold simulation models performed in this study show that, at different human posture (depending upon knee bending angle), the stress experienced on the LM was found to be different. Thus, from the selected biomaterial scaffold system, silicone was found to be better. This study demonstrates how to develop scaffolds with sufficient mechanical property to withstand the force exerted in the different angles. Similar observations were also reported by Pena et al.[24] They developed a finite-elemental analysis to study why lateral meniscectomy is more dangerous than a medial meniscus in a fully extended knee. They found that, after LM meniscectomy, maximum shear stress exerted in articular cartilage was found to be 200%. In this study also from the statistical analysis, we proved that LM tear incidence was higher than MM tear. Thus, this finding also suggests that a proper tissue engineering strategy for such damages is need of hour. Therefore, before developing knee meniscus scaffolds for tissue engineering application, simulation study can give an insight for the development of implantable scaffolds.

5 Conclusion

This study focuses on how to implement a better tissue engineering strategy in a systematic way starting from the problem definition to simulation studies for the development of a potential implantable scaffold for knee meniscus injury. As observed in the studied population, road traffic accidents are the second major cause of meniscal injuries in India. Based on these results, we suggest that the medium active or less population when they start recreational sports activities without proper training may have a high risk of meniscus injuries, due to change in lifestyle pattern. Thorough study on severity of meniscus injury and simulation studies can pave way for development of implants for knee meniscus injuries. This statistical study clearly indicates that the problem of meniscal injury is predominant among the south Indian population. Hence, there is a need for appropriate treatment strategy that can be used to address the said meniscal problem and to replace the damaged meniscus whenever possible which in turn would prevent further deterioration of the joint.

Publisher's Note

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Compliance with ethical standards

Conflict of Interest

All authors declare that they have no conflict of interest.

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